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THE REDUCTION DIVISION IN FUCHSIA*.

Blanche McAvoy.

The genus Oenothera has been of great interest in recent years to biologists because of DeVries' studies on Oenothera lamarckiana in connection with his development of the mutation theory. A number of investigators have worked on Oenothera among whom may be mentioned Lutz (13 & 14), Geertz (10), Gates (5, 6, 7, 8 & 9), and Davis (2 & 3).

Some of the investigators have worked on the cytology of the micro- and mega-sporocytes while others have worked on the genus from a somewhat different point of veiw, getting the complete life history as a basis for evidence of the validity of DeVries' results.

Geertz (10) has made a complete study of Oenothera lamarckiana beginning with the archesporial cell, taking up the cytology and continuing on through the details of the complete life history. In some of the microsporocytes he describes threads with small chromatin discs on them, some of the threads being quite small and others thicker. He calls the early contraction generally observed in prepared sporocytes synapsis and says that in some cases there were loops extending out from the contracted mass. The material may be contracted around the nucleolus or may be separated from it. Immediately after synizesis he represents fully formed chromosomes in the nuclear cavity. He says that the 2x number of chromosomes were formed and later

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united in pairs. He did not find a conjugation of two threads during synapsis. He mentions a slight resting stage between the first or heterotypic division and the second, homotypic division, but does not speak of any longitudinal splitting of the heterotypic chromosomes until after the transverse splitting occurs. The longitudinal splitting was visible just before the chromosomes reached the poles.

Gates (7) in his paper on Oenothera rubrinervis, states that the contraction of the chromatin material is synapsis and that since the cytoplasm of the cells shows no contraction, the cell is perfectly fixed. For this reason, the contraction, so constantly observed at an early stage in the process, is not an artifact, but is a real contraction stage, leading to synapsis. As this contraction proceeds the reticulm is re-arranged into a long, continuous delicate thread. No indication of a doubling or pairing of the threads was evident. After the synapsis the spirem shortens and thickens and begins to arrange itself more loosely in the nuclear cavity. This shortening is progressive and continues for some time. He states that the shortening may be uniform, or it may vary or may be irregularly constricted at varying intervals. This thickened thread now segments transversely into fourteen chromosomes—fourteen being the sporophytic number. Then these chromosomes break up into pairs which later fuse with each other leaving the x number of chromosomes. They are taken on the spindle and reduction follows in the usual way.

In his paper on Oenothera lata x O. gigas (9) he begins his dicussion with the telophase The usual number of chromosomes found in the hybrid is twenty-one, seven being of maternal and fourteen of paternal origin. In the reduction one germ cell receives ten and the other eleven chromosomes. In a few cases nine and twelve chromosomes were the numbers found at the respective poles. One cell was found which had twenty chromosomes, ten of which went to each pole. The segmentation into ten and eleven proves that there is not a pairing of homologus chromosomes of maternal and paternal origin but the segmentation tends to be into two numerically equal parts. Gates claims evidence from his work, that there are two general methods of chromosome reduction, one a side to side pairing of chromatin threads (parasynapsis) to form a double spirem; the other involving an end to end arrangement (telosynapsis) of maternal and paternal chromosomes, to form a single spirem which afterwards splits longitudinally. He says an individual always has as many chromosomes as the sum of the chromosomes in the germ cells which go to form the new plant. This fact, he says, supports the genetic continuity of the chromosomes. He has not shown whether the chromosomes have equal or unequal hereditary value.

In his paper on Oenothera lata x O. lamarckiana (5) he finds starch grains present in the cytoplasm of the mother cell. These grains become more abundant until the reduction takes place after which they are not found. He finds what he calls "pro-chromosomes", but in a later paper considers these bodies to be nucleoli. The presynaptic stages show a continuous spirem which is exceedingly delicate and coiled. Then follows the contraction, after which follows a stage in which the spirem is much shortened and several times thicker than just before the contraction. He does not believe that the nucleoli break up and move out into the cytoplasm waiting to be re-collected into a new nucleolus in the new nucleus, as Schaffner believes to be the case in Lilium philadelphicum. There are twelve chromosomes and one or two "heterochromosomes", or as he calls them in a later paper—nucleoli. He believes O. lamarckiana to be a pure strain and not a hybrid.

In the paper on Oenothera gigas (8) Gates says "The absence or partial absence of a close pairing of chromosomes in diakinesis and on the heterotyptic spindle is in strong contrast to the condition in other genera of plants where the chromosomes are regularly paired. However * * * similar failure to pair is often exhibited. These cases appear to be the exceptions to the general law enunciated by Montgomery in 1901 from his observations on Hemiptera, that homologous chromosomes of maternal and paternal origin pair with each other in synapsis. Later observations on a variety of forms, in which there are morphological chromosome differences, show that ordinarily chromosomes of similar size and shape pair with each other and justify the view of Montgomery which has been widely adopted. There is some variety in the size and shape of the chromosomes but nothing constant was observed." In the anaphase the longitudinal split generally described does not always show. There are some irregularities in the reduction such as an unequal number of chromosomes passing to the two poles.

Davis in his first paper on Oenothera (2) observes the nucleus filled with a close reticulum having the chromatin material distributed around the periphery of the nucleus. Next follows the appearance of extremely delicate strands connecting the bodies, forming an open net work. These strands readily thicken and become more numerous until finally the nucleus is filled with a relatively close reticulum. During this time the chromatin bodies become smaller and seem to contribute their material to formation of the strands so that the only large structures in the nuclei are the nucleoli. Synapsis, as he calls the synizetic contraction, begins slowly and finally carries the strands away from the nuclear wall. During this contraction there is a marked change in the structure of the reticulum At first the reticulum

was connected at many points but in time it is easily seen that a true spirem is being formed. During the main part of the contraction the knot is so close that it is difficult to see any of the structure. Usually there are threads sticking out at the edge of the knot. During the process the thread of the spirem is shortened. He thinks the contraction is due to this shortening of the threads of the reticulum as it goes to make up the spirem. The forms of the "synaptic" knot are varied. When the knot loosens the chromatin material is seen to be in the form of seven bivalent chromosomes, which have assumed the form of rings some being linked together. These rings, he says, remain together until the two halves of the bivalent rings are pulled apart on the spindle.

In the second paper(3) he states definitely that he believes the dark staining masses formed on the perifery of nucleus and connected with one another by delicate threads to be the "prochromosomes" described by Overton (17) but he says there is no evidence that they are arranged side by side in pairs on a system of threads that might be interpreted as two parallel spirems. The chromatin bodies are scattered throughout the nucleus but where ever there are two together they lie end to end upon a delicate strand that runs in the direction of the longer axes. The nucleus after considerable time, becomes filled with a close reticulum at which stage the chromatin bodies can only be distinguished with difficulty. He found some differences in the method of chromosome formation from that described in his first paper. The knot loosens and a shorter thicker thread emerges. This spirem is then constricted into a chain of fourteen chromosomes. A longitudinal split becomes apparent just before the heterotypic chromosomes reach the poles.

In Erythronium, Schaffner (19) finds the chromatin material going into synizesis—a term used to designate the contraction as being a separate thing from the fusion of the chromatin. This contraction he considers to be an artifact. After synizesis he finds the formation of a spirem which by twisting, forms loops all around the nuclear wall. There then occur breaks between the loops. The loops continue to twist until the chromosomes are fully formed. The chromosomes are described as having quite distinctive shapes.

In Lilium tigrinum (20) he found a continuous spirem with a single row of chromatin granules. This spirem enters synizesis and comes out of it without a conjugation or a division of granules. Later on the granules divide but the linen thread does not show a distinct separation. The continuous spirem shortens, thickens, and twists into twelve loops which break into twelve chromosomes. These chromosomes are attached to the spindle fibres in the mother star at or near the end and during the reduction the chromosomes uncoil and separate by a transverse division at the

middle. The next division is longitudinal and the resulting nuclei form the tetrads. The nucleoli fragment and pass into the cytoplasm during both the first and second divisions.

In Agave viriginica (22) Schaffner described bivalent protochromosomes which formed a long delicate continuous spirem with a single row of chromatin granules. Synizesis follows during which there is no union of the spirems. A study of living material did not show any noticeable contraction of the nuclear contents and the nucleoli were usually found occupying a central position in the nucleus. While the chromatin granules undergo transverse division, the spirem shortens and thickens and then twists up into twelve loops of different size and shape which are pressed against the nuclear wall. These loops then break apart into four large, three ring shaped and five small irregular chromosomes. One or two nucleoli are present which may be thrown out into the cytoplasm. The spindle is bipolar and at the first division the chromosomes divide transversely but the second division is longitudinal.

Miss Hyde in her paper on Hyacinthus (11) did not find definite protochromosomes nor a splitting of the spirem. She observed a continuous spirem which formed eight definite loops. These loops break apart and form eight chromosomes of different characteristic sizes and shapes.

There has been much controversy as to the real nature of the contraction generally observed in the early stages of the division of reduction cells. In most cases this contraction is accompanied by an enlargement of the nucleus due to the expansion of the nuclear wall. McClung (15) has suggested the term synizesis, to be used to mean the contraction as distinct from synapsis, synapsis being restricted to the fusion of simple chromosomes into multiple ones. Lawson (12) has recently investigated the problem and his interpretation of synizesis is simply that it represents a growth period of the nucleus—a period during which there is a great increase in the amount of nuclear sap, which results in a distention and withdrawal of the nuclear membrane from the chromatin. As to why the nucleus should swell so much immediately before the reduction division he answers as follows. Each cell is charged with sufficient food substance for the production of the tetrad. Moreover there are two divisions which follow one another in quick succession. The pressure of the cell sap acting from within causes the nuclear membrane to distend and the nuclear cavity to expand. The expansion is at first gradual and continuous until the nuclear cavity grows to twice or even three times its original size.

As the growth proceeds the membrane is gradually withdrawn from the chromatin mass within. The result of this withdrawal of the nuclear membrane is the formation of a large clear area of

nuclear sap containing the mass of chromatin which has been left to one side. No evidence whatever was found to show that any contraction of the chromatin had taken place. The enlargement of the nuclear cavity and the consequent withdrawal of the membrane away from the chromatin gives the appearance of a contraction, but actual measurements failed to show any diminution in chromatin area.

During this stage definite changes take place in the nature of the chromatin threads as the spirem becomes differentiated.

This view is somewhat similar to that expressed by Schaffner in Synapsis and Synizesis (21) although he believes that synizesis is a true shrinking of chromatin material due to the effect of the killing fluid on the nuclear contents, which has become loosened from the nuclear wall on account of the swelling of the nucleus. One important proof for this conclusion was the fact that in many instances there are symmetrical contractions showing the same peculiarities as in ordinary plasmolysis. On the other hand Gates and Davis whom I have quoted above believe this stage to be natural and do not believe it to be an artifact. They base their opinion on the fact that the contraction is of such constant occurrence in all forms studied.

Because of the apparently peculiar process of chromosome formation reported for the Oenotheras differing somewhat from both the type of division held by Allen and others on the one hand and by Schaffner and others on the other, it seemed desirable to the writer to investigate the formation of the chromosomes in Fuchsia. Accordingly a study of the reduction division in the microsporocytes of Fuchsia was taken up under the direction of Professor John H. Schaffner, whose help and kindly criticisms have been of inestimable value throughout the whole year's work.

When starting the problem I expected to find the chromosomes formed in the manner described by Gates for Oenothera rubrinerves and by Davis for Oenothera biennis. The most of my attention was directed toward finding how the chromosomes were formed. I wished to see whether it was by the thickening of the spirem followed by a transverse division by which the 2x number of chromosomes were differentiated and then cut off in pairs, which should afterward fold together to form the bivalents; or whether the spirem thickened and then folded and twisted around into the number of loops before a subsequent separation into the reduced number of chromosomes. These two methods are very similar in results but somewhat different in detail. Gates found the chromosomes formed in the first way described and since Fuchsia is not so distantly related to Oenothera, it was natural to expect to see the chromosome formation brought about in much the same way that Gates found in Oenothera.

Two varieties of the Fuchsia commonly grown in greenhouses were used. Both were varieties of Fuchsia speciosa (Hort.), of rather small size—one variety having red and the other white sepals. The species is commonly supposed to be a hybrid. Fuchsia speciosa was obtained from the greenhouse in connection with the Botany building of the Ohio State University at Columbus The buds which showed the reduction stages were quite small, being about 3-5 mm. in length. They were killed in Schaffner's weaker chrom-acetic solution. Material was left in the killing fluid for 24 hours and then thoroughly washed and run up to 70 per cent alcohol where it was left for several days. Then 85, 95 and 100 per cent alcohols were added in turn and chloroform and from that the buds were slowly taken into pure parafin and imbedded. Sections were 10–15 mic. thick. Delafield's Haemotoxylin was tried with poor success. The best stain was a combination of Safranin and Iron Haemotoxylin. The slides were transferred from 25 per cent alcohol to Safranin and left for four hours. They were then washed off in 25 per cent alcohol and put into water and then transferred to iron alum. Slides were kept in iron alum for four hours and then washed for a while in water, after which which they were left over night in Haemotoxylin. Next day the slides were bleached in iron alum, and in some cases acid alcohol, and were mounted in balsam.

The tapetal layer is rather slow in developing but by the time the sporocytes began to be differentiated it can easliy be distinguished as a limiting layer of the sporogenous tissue. The sporogenous tissue remains intact during all the early stages of the reduction process and it is only while the chromosomes are being formed that the sporocytes become separated from each other and from the tapetal wall. In cross section the stamens show the usual four microsporangia and each cavity usually contains from five to eight sporocytes. As the stamen grows older the number of sporocytes, that show in cross section decreases until four is the more usual number. This may be due to the rapid elongation of the anther at the time when the sporocytes are separating.

The nucleus in the early stages is rather small and is made up of a reticulum, containing dark staining masses (Fig. 1). As the nucleus enlarges these lumps become much more prominent and definite and may be regarded as protochromosomes (Figs. 2, 3, 4). In no case was it possible to make a positive count of these masses since some of them had apparently begun to disintegrate while others were just forming. As the lumps disappear the material seems to go toward the formation of small chromatin granules which are scattered along a delicate thread (Figs. 4, 5, 6). This thread could be traced for some distance in a number of the cells. Often there are two nucleoli present in one nucleus but in most

cases there is only one. There is no difficulty in distinguishing the nucleoli from chromatin material since the safranin used in the combination stain gives the nucleoli a peculiar reddish tinge while chromatin material stains nearly black The nucleolus is in the middle of the nucleus, sometimes a little to one side; and on the periphery of the nucleus is the network and chromatin granules spoken of above. A little later the nucleus begins to swell very considerably, and gradually the network is loosened in one place or another from the nuclear wall. At this time the nucleolus is still in the middle of the nucleus. As the process continues the nucleus becomes larger and more of the threads becomes loosened from the wall (Figs. 6. 7. 8).

At this stage the synizesis begins, the spirem massing together into an irregular lump which may or may not enclose the nucleolus. In some cases the nucleolus may be entirely separate from the synizetic knot. No division of the granules or longitudinal split of the spirem was observed. There is a well defined thread now present and in some cases loops of the thread could be seen sticking out from the opaque knot (Figs. 9 and 11). In other places little apparently free ends of the thread projected from the mass. On these threads definite chromatin granules were plainly visible and could easily be counted in any free loop. Whether the free ends represented natural breaks in the spirem or injuries caused by the contraction or the cutting, could of course, not be determined. But the appearance of the spirem before and after the synizesis indicates that the spirem is continuous. The contracted chromatin mass was sometimes formed to one side of the nuclear cavity and sometimes in the middle (Figs. 9, 17). Sometimes it extended across the nucleus. There were various stages of contraction from the loosely coiled mass in which the threads were clearly visible (Fig. 11) to the tightly contracted mass in which no structure, whatsoever, could be made out. During older stages of the synizesis the knot is very much looser and the thread is much more complete and is thicker with the granules of a more uniform size. There is no question but that there has been a contraction of the chormatin, the mass occupying a much smaller area than before, while the nuclear cavity is much larger. Whether some of the enlargement of the nuclear cavity was due to plasmolizing reagents or entirely due to a normal growth could not be determined since there is considerable difference in the size of various nuclei of apparently the same stage of development.

Immediately after the synizesis the threads are delicate and contain numerous small granules. It is exceedingly difficult to follow the thread through all its convolution but in some cases it could be traced for quite a long distance (Figs, 12, 13, 14). There is generally one nucleolus at this stage but in some cases two are

to be seen. As division advances the thread continues to elongate up to a certain stage when it is rapidly thrown into loops (Figs. 12, 13, 14), and begins to shorten and thicken constantly until the chromosomes are fully formed (Figs. 13, 15, 17, 21, 22). In the very earliest stages of the looping (Figs. 13, 14, 15, 16) the spirem may be traced for a long distance and the loops are found on the upper and lower surface of the nuclear wall showing that the loops are formed along the periphery of the nucleus and not as loops sticking out from a synaptic knot into the nuclear cavity.

However, in the earlier stages there is a considerable crossing of threads in the center. Figure 13 shows four or more well defined loops already formed. These loops and the thread of which they are formed are still rather delicate. The nucleolus is in the center of the nuclear cavity. In some cases the cell wall begins to become somewhat indistinct at about this stage, (Fig. 12), but in others the wall remains well defined until the tetrads are fully formed inside the original cell. In most cases the sporocytes have not separated from each other nor from the tapetal layer, and have in consequence, not yet rounded up. The loops of the thread are formed in just such a way as loops would be formed in a heavy string if two ends of the string were held between the fingers and then twisted; twisting both ends in opposite directions. Some of these loops showed more than one twist. As the loops become tighter the spirem often appears as though it contained prominent knots. The granules are still very evident on the spirem where much looping has taken place but at this stage no doubling was visible. This does not necessarily indicate that division has not taken place; the granules may be lying too close together to be separated with the magnification used, or the differentiation possble with the sarfanin-haemotoxylin stain. As the looping proceeds the granules become less and less prominent until on the fully formed loops no granules are to be seen (Figs. 15, 16, 17). The loops finally break apart to form the bivalent chromosomes (Fig. 17). While chromosome formation is going on the nuclear cavity is apparently still enlarging (Figs. 15–19), but later as the nuclear wall disappears, the cytoplasm encroaches rapidly and fills the area around the contracting group of chromosomes (Figs. 20–24). Just about this time the sporocytes begin to separate from each other and assume a more rounded shape and the nuclear wall becomes more delicate. When all the loops are formed they lie around the periphery of the nucleus and can readily be seen and counted by focusing up and down. In each case the drawings were made from cells whose complete nucleus showed and had been undisturbed in the cutting. It was somewhat difficult to draw correctly those loops which were to the side of the nucleus where it was often impossible to see the actual shape. In some cases the ends

where the break occurs become fastened together, making somewhat irregularly shaped rings, some of these rings having little loops in them (Figs. 18b, 19).

In other cases, after the break occurred, the ends of the loops did not fuse, but projected as free limbs (Fig. 19). There was a great deal of difference in the newly formed chromosomes. In the figures, all the chromosomes are shown in one plane as projections, but under the microscope they were more easily distinguished and the details could be more easily traced out by focusing. The large chromosomes in the middle of Figure 18 which overlap are figured separately to show their actual form; 18a being the one on top and 18b the one below (Figs. 18a, 18b). Figures 17, 19, 18, 20, and 21, show the chromosome differences plainly; six are quite large, six small and two of intermediate size. The difference in shape is well shown in Figures 19 and 20. In the earlier stages it can easily be seen that some of the chromosomes have not doubled up nor formed complete rings. Gradually all of them twist up tighter until most of the chromosomes appear as small irregular masses (Figs. 21, 22). The nuclear wall has practically disappeared by the time the chromosomes have fully contracted (Figs. 21, 22, 23). By this time, also, the sporocyte has rounded up and withdrawn from the neighboring cells. The cytoplasm appears spongy and, in most cases, is withdrawn from the cell wall. The nucleolus seems to disappear at about the time the nuclear membrane becomes indistinct. What becomes of it was not determined but in some cases nucleolus-like masses were seen in the cytoplasm. As will appear from the above description and consideration of the figures presented, it becomes evident that the details of chromosome formation in Fuchsia does not agree with that of Oenothera as described by Gates, Davis and Geertz. The loops are formed from a very slender spirem and no thickening into a chain of univalent chromosomes is apparent. The incipient loops before the separation occurs are quite distinct and these loops were followed through their development and gradual transformation until the fully formed bivalent chromosomes were present. Although the behavior of the spirem is somewhat different from that reported for Oenothera the final result is identical. The spirem breaks up apparently into chromosome pairs which, coming to lie side by side, by folding and twisting together are transformed into bivalents in the same manner as described by Gates. The bivalents are formed by an end to end fusion and subsequent folding of pairs of univalents. The number of chromosomes could be counted in ten or twelve preparations and each count was fourteen. Figures 22 and 23 show the fully formed chromosomes before the formation of the spindle. In Figure 22 one of the large chromosomes lies out separate from the rest and all except this large one are

somewhat connected by delicate strands of material. The separation of the large chromosome from the rest may have no special significance for later all fourteen seem to be connected. The connecting strands are not evident until after the chromosomes are fully formed but appear before the spindle. In Figure 23 the connection is very distinct and the appearance is much like what Gates has shown in some of his figures.

The nuclear wall has entirely disappeared by this time and the cells are spherical. The fully formed chromosomes are of rather indefinite shape although there is a difference in size, but there is no such characteristic shapes as found by Schaffner in Lilium tigrinum and Erythronium and by Miss Hyde in Hyacynth. However, the peculiarities of size and form noted earlier are still in evidence (Fig. 24).

While the chromosomes are scattered in the nuclear cavity the delicate strands of material that connect them seem to draw them up closer into the central part of the nuclear area.

The sections were not stained with the special object of studying the spindle but when it became evident it was a bipolar structure within the nuclear cavity and the chromosomes were apparently attached to the delicate fibers. At this stage the cytoplasm has usually penetrated into the nuclear area and surrounds the spindle but occasionally the preparations show a clear surrounding space which may be due to plasmolysis (Fig. 25).

In Lilium tigrinum Schaffner found that the chromosomes in the reduction division were fastened to the spindle near the end and that as the chromosomes were pulled toward the poles the break occurred transversely causing one of the univalent chromosomes to go to each pole. In Fuchsia the chromosomes are so small and compact that no ends can be seen sticking out from the apparently homogenous mass. Even with a magnification of 2500x the chromosomes seemed perfectly homogenous. As the chromosomes are drawn into the equatorial plate they still retain their individuality and can be counted without great difficulty. At this point they are hard to draw due to the fact that they lie under each other and can be seen best by focusing.

The main purpose of the investigation was to study the formation of the bivalent chromosomes but a series of older sections brought out another point of interest that might be mentioned. There are irregularities in the development of the tetrads which may be significant in connection with the supposed hybrid nature of our greenhouse varieties of Fuchia. In some cases normal tetrads were formed, in others as high as six to eight nuclei of various sizes were observed in one sporocyte (Figs. 33, 34). This condition has been known for some time. In 1886 Wille (23) reported that he had observed as high as eight cells developed from the pollen-mother cells of Fuchsia. The same condition was

observed in Hemerocallis by Fulmer (4). In some cases the cytoplasm between two newly formed nuclei was separated although no visible wall was formed; but in most cases the nuclei were formed and remained imbedded in the general cytoplasm (Figs. 28–34). In one case a regular tripartite arrangement of three nuclei was observed and these were surrounded by separate masses of cytoplasm (Fig. 31). In none of these cases was the original sporocyte wall disintegrated. The further development of the smaller nuclei was not studied although that might be an interesting investigation.

SUMMARY.

1. In the reduction division of Fuchsia speciosa there is apparently an end to end fusion of the univalent chromosomes, forming a continuous spirem which twists and folds up into a definite number of loops which represent the incipient bivalent chromosomes, fourteen in number.

2. The loose network of the resting nucleus at an early stage begins to show a massing of chromatin material into indefinite lumps of approximately the reduced number of chromosomes. These masses probably represent the arrangement of the chromatin into a definite mosaic, preparatory to the synaptic conjugation of the univalent into the bivalent chromosomes.

3. Gradually the lumps disappear and the material seems to go toward the formation of prominent granules that arrange themselves along a delicate thread.

4. Next follows a period during which there is an evident swelling of the nucleus. In consequence of this swelling the threads are pulled loose or withdrawn from the nuclear wall, and the chromatin material collapses in a mass. It may collapse around the nucleolus or to one side of it, or it may collapse so that the nucleolus has no connection with it. The contracted portion may lie in the center of the nucleus or in contact with the nuclear wall. This synizesis is regarded as an artifact although no definite evidence was obtained for or against this supposition.

5. After the synizesis the spirem is apparently continuous and the granules appear small and evenly distributed throughout its length. At first there is little or no looping but soon the spirem begins to show that it is laid in delicate little loops. The loops are arranged on the inside of the nuclear wall. In some sporocytes as high as eight loops could be determined at a rather early stage, still more or less connected, but by the time the fourteen loops are fully formed they are usually broken apart.

6. After breaking apart the loops thicken and tighten until masses of various sizes and shapes were formed, four being quite small and five of rather large size.

7. The fully formed chromosomes are then seen to be connected by delicate strands. About this time or a little before the nuclear wall has disappeared.

8. The chromosomes are taken on to a bipolar spindle and gradually pulled into the equatorial plane.

9. There is an irregularity in the formation of the microspores. Frequently as high as eight are formed from one sporocyte.

LITERATURE CITED.

1. ALLEN, C. E.—Nuclear Division in the Pollen Mother-cells of Lilium canadense. Annals of Botany, **19:** 189–258, 1905.
2. DAVIS, B. M.—Cytological Studies in Oenothera I. Pollen Development of Oenothera grandiflora. Annals of Botany, Vol. XXIII, No. 96, 1909.
3. DAVIS, B. M.—Cytological Studies of Oenothera II. The Reduction Division of Oenothera biennis. Annals of Botany, Vol. XXIV, No. 96, 1910.
4. FULMER, E. L.—The Development of Microsporangia and Microspores of Hemerocallis fulva. Botanical Gazette, **28:** 81–88, 1899.
5. GATES, R. R.—Pollen Development in Hybrids of Oenothera lata x O. lamarckiana, and its Relation to Mutation. Botanical Gazette, **43:** 81–115, 1907.
6. GATES, R. R.—Hybridization and Germ cells of Oenothera Mutants. Botanical Gazette, **44:** 1–2, 1907.
7. GATES, R. R.—A Study of Reduction in Oenothera rubrinervis. Botanical Gazette, **46:** 1-34, 1908.
8. GATES, R. R.—The Stature and Chromosomes of Oenothera gigas, DeVries. Archiv. fur Zellforschung. 3 Band, 4 Heft, 1909.
9. GATES, R. R.—The Behavior of Chromosomes in Oenothera lata x O. gigas. Botanical Gazette, **48:** No. 3, 1909.
10. GEERTZ, J. M.—Beitrage zur Kenntnis der Cytologie und der partiellen Sterelitat von Oenothera Lamarckiana. Recueil des Travaux Botaniques Neerlandais, Vol. V, sg 3, 1908.
11. HYDE, EDITH—Reduction Division in Hyacinthus. Ohio Naturalist, Vol. IX, No. 8, 1909.
12. LAWSON, A. A.—The Phase of the Nucleus Known as Synapsis. Trans. Roy. Soc. Edinburg, Vol. XLVII, Part III, No. 2, 1911.

13. LUTZ, ANNE H.—Preliminary Notes on the Chromosomes of Oenothera lamarckiana and One of its Mutants, O. gigas. Science, N. S. 26: 151–152, 1907.

14. LUTZ, ANNE H.—Chromosomes of the Somatic Cells of the Oenotheras. Science N. S. 27: 335, 1908.

15. MCCLUNG, C. E.—The Chromosome Complex of Orthopteran Spermatocytes. Biol. Bull. 9: 304–340, 1905.

16. MONTGOMERY, T. H.—A Study of the Chromosomes of the Germ Cells of Metazoa. Trans, Am. Phil. Soc. **20:** 154–236, 1901.

17. OVERTON, J. B.—Ueber Reduktionsteilung in den Pollenmutterzellen einiger Dikotylen. Jahrb. Wiss. Bot. **42:** 121–153, 1905.

18. SCHAFFNER, J. H.—Contributions to the Life History of Lilium philadelphicum III. The Division of the Macrospore Nucleus. Botanical Gazette, **23:** 430–449, 1897.

19. SCHAFFNER, J. H.—A Contribution to the Life History and Cytology of Erythronium. Botanical Gazette, **31:** 369-387, 1906.

20. SCHAFFNER, J. H.—Chromosome Reduction in the Microsporocytes of Lilium tigrinum. Botanical Gazette, **41:** 183–191, 1906.

21. SCHAFFNER, J. H.—Synapsis and Synizesis. Contribution from the Botanical Laboratory of the Ohio State University, 1907. Ohio Naturalist, Vol. VII, No. 3.

22. SCHAFFNER, J. H.—The Reduction Division in the Microsporocytes of Agave virginica. Botanical Gazette, **47:** 190-214, 1909.

23. WILLE, N.—Uber die Entwickelungsgeschichte der Pollenkorne der Angiospermen und das Wachsthum der Membranen durch intussusception. Christiania, 1886.

EXPLANATION OF PLATES I AND II.

The plates are reduced $\frac{5}{8}$ in reproduction. All the figures were drawn with a conpensating ocular 18, and an oil immersion 1/12, which makes a magnification of about 2500.

PLATE I.

Fig. 1. Microsporocyte showing the resting chromatin network.

Fig. 2. Microsporocyte showing the chromatin material beginning to collect in little lumps.

Fig. 3. Later stage showing the further development of the chromatin masses.

Fig. 4. Microsporocyte showing well formed masses with more prominent connections.

Figs. 5, 6. Microsporocytes still showing the larger chromatin masses but having well formed linin threads on which are seen chromatin granules.

Figs. 7, 8. Microsporocytes showing the spirem with granules on it and still showing some larger masses of chromatin material.

Figs. 9, 10, 11. Microsporocytes showing the chromatin material in various stages of synizesis.

Fig. 9. The microsporocyte in contraction showing a few strands on which granules may be distinctly seen.

Fig. 12. Sporocyte with the spirem well formed, showing a slight disposition to loop.

Fig. 13. Sporocyte showing well formed loops.

Fig. 14. Sporocyte showing the loops well formed.

Fig. 14a. Part of the looped spirem from Fig. 14, showing the small granules on the spirem and in one case there are two loops fastened together.

Fig. 15. Sporocyte showing that the loops have become tightened.

Fig. 16. Sporocyte showing loose and tight loops.

Fig. 17. Sporocyte in which the loops have separated from each other and show characteristic sizes and shapes.

PLATE II.

Fig. 18. Later stage of Fig. 17 in which some of the loops have become tighter.

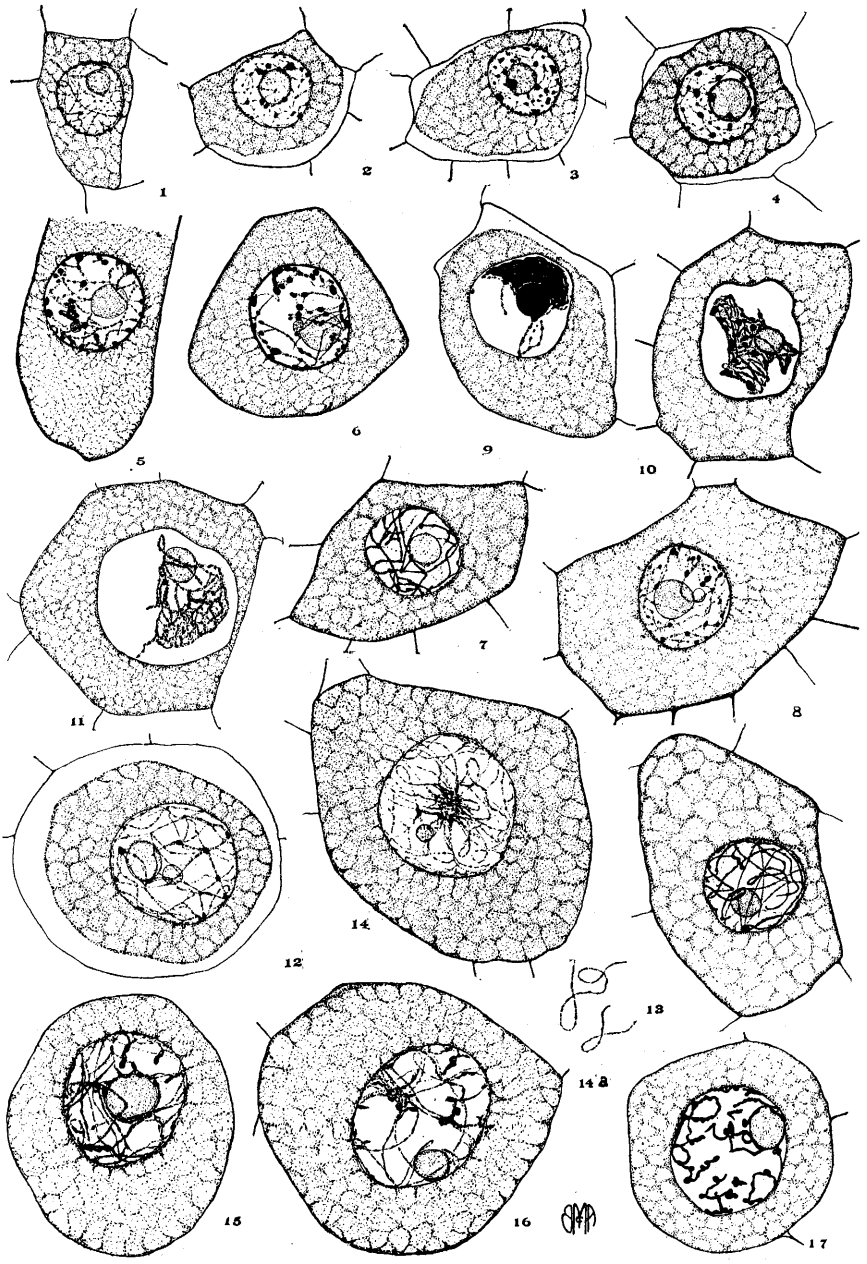
Fig. 18a. A loop from the top of the nucleus shown in Fig. 18.

Fig. 18b. A loop from the bottom of the nucleus in Fig. 18.

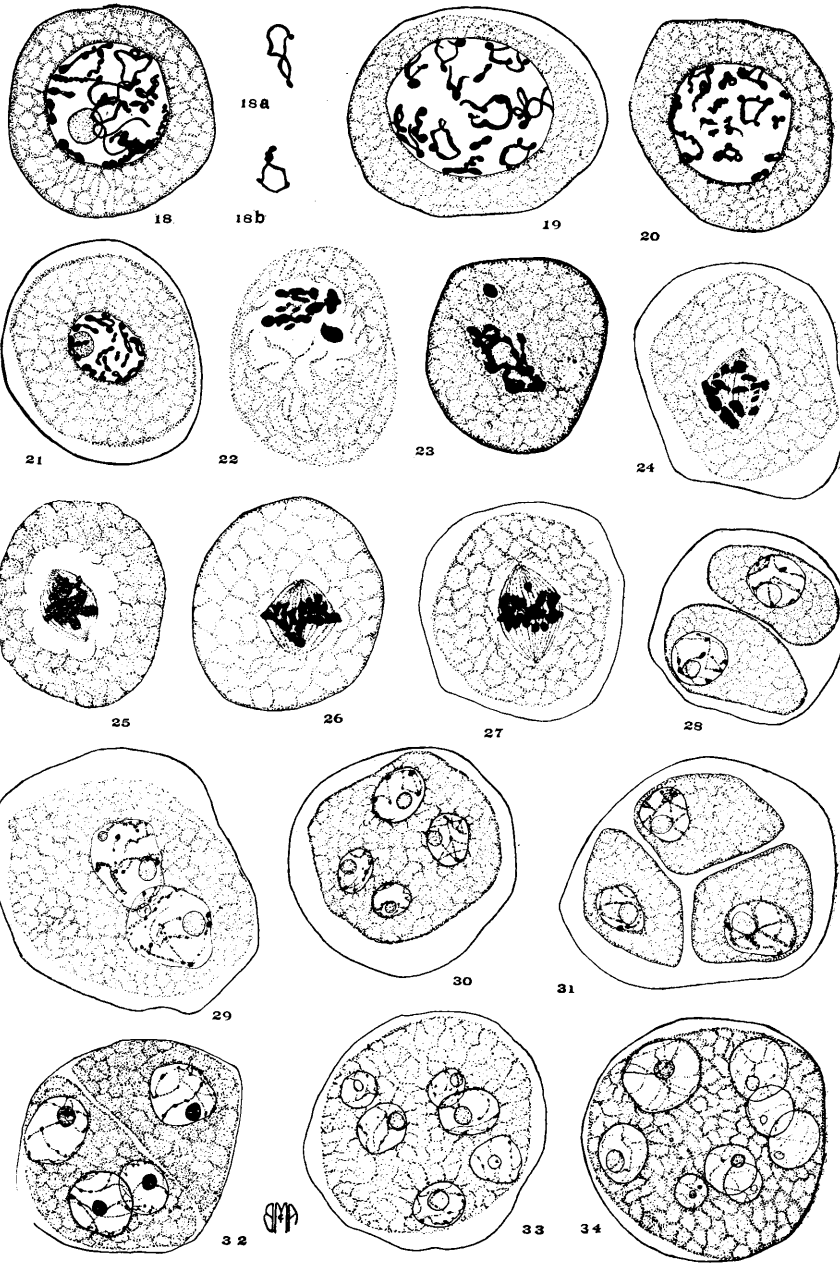
Fig. 19. Chromosome loops of various characteristic sizes and shapes. The nucleolus has disappeared.

Fig. 20. Later stage in the formation of the chromosomes. The loops are tightening.

Fig. 21. The loops have become tighter and have come to lie closer together.



McAvoy on "Reduction Division in Fuchsia."



McAvoy on "Reduction Division in Fuchsia."

Fig. 22. The chromosomes are shown lying in the nuclear area. Delicate connecting fibers are seen connecting the chromosomes.
Fig. 23. Chromosomes are seen connected by delicate strands and the cytoplasm has penetrated into the nuclear area.
Fig. 24. Chromosomes on the bipolar spindle.
Figs. 25, 26. Chromosomes on the spindle.
Fig. 27. Chromosomes near the equatorial plate.
Fig. 28. Two nuclei surrounded by cytoplasm inside the original sporocyte wall.
Fig. 29. Two nuclei imbedded in the cytoplasm of the original sporocyte.
Figs. 30, 32. Four nuclei in the cytoplasm of the original sporocyte.
Fig. 31. Three nuclei in three masses of cytoplasm inside the original sporocyte wall.
Fig. 33. Sporocyte wall and cytoplasm in which six nuclei are imbedded.
Fig. 34. Eight nuclei following the process of reduction. The cytoplasm has not begun to separate.